Liquefaction, Saccharification, and Fermentation of Ammoniated Corn to Ethanol

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Treatment of whole corn kernels with anhydrous ammonia gas has been proposed as a way to facilitate the separation of nonfermentable coproducts before fermentation of the starch to ethanol, but the fermentability of ammoniated corn has not been thoroughly investigated. Also, it is intended that the added ammonia nitrogen in ammonia treated corn (~1 g per kg corn) may satisfy the yeast nutritional requirement for free amino nitrogen (FAN). In this study, procedures for ammoniation, liquefaction, saccharification, and fermentation at two scales (12-L and 50-mL) were used to determine the fermentation rate, final ethanol concentration, and ethanol yield from starch in ammoniated or nonammoniated corn. The maximum achievable ethanol concentration at 50 h fermentation time was lower with ammoniated corn than with nonammoniated corn. The extra nitrogen in ammoniated corn satisfied some of the yeast requirements for FAN, thereby reducing the requirement for corn steep liquor. Based upon these results, ammoniation of corn does not appear to have a positive impact on the fermentability of corn to ethanol. Ammoniation may still be cost effective, if the advantages in terms of improved separations outweigh the disadvantages in terms of decreased fermentability.

Keywords: bioethanol, pretreatment, anhydrous ammonia, free amino nitrogen (FAN), yeast nutrients

Introduction

The production of fuel ethanol from corn may be improved through the recovery of more valuable coproducts, thereby lowering the net feedstock cost. In the dry-grind fuel ethanol industry, coproducts such as distillers' dried grains and carbon dioxide are obtained after liquefaction (conversion of starch to soluble form), saccharification (conversion of soluble starch to glucose), and fermentation (conversion of glucose to ethanol). In corn wet milling, coproducts such as corn oil, corn gluten meal, and corn gluten feed are recovered before fermentation. Through continued research and development, the cost of ethanol production from corn starch may be lowered by recovering new coproducts and by establishing new fermentation-based process technology.

The production of value-added coproducts depends on the efficient separation of the different parts of the corn kernel. Such fractionation is facilitated by first softening the dry corn by soaking (tempering) in a liquid such as water. However, the cuticle and pericarp (hull) form an effective barrier to the penetration of liquids. Steeping in water, as in corn wet milling, can take 36 h or longer.[1] Alternatively, the hull can be removed by alkaline debranning, whereby hot caustic solution is used to loosen the hull, followed by mechanical separation. However, this process is too costly for fuel ethanol production as the bran has a low market value.[2]

Treatment with anhydrous ammonia gas may be a cost effective alternative to alkali debranning. Rapid absorption of the moisture (10–15%) in corn leads to very short exposure times, and thus to small equipment and lower capital cost. In laboratory tests, corn that retained less than 0.1% by weight of ammonia nitrogen after treatment could be mechanically fractionated more readily than untreated corn.[3] Added ammonia can also supply the yeast nutritional requirement for free amino nitrogen (FAN).[4] The recovery of corn bran and other coproducts before fermentation can increase the efficiency of the fermentation, because

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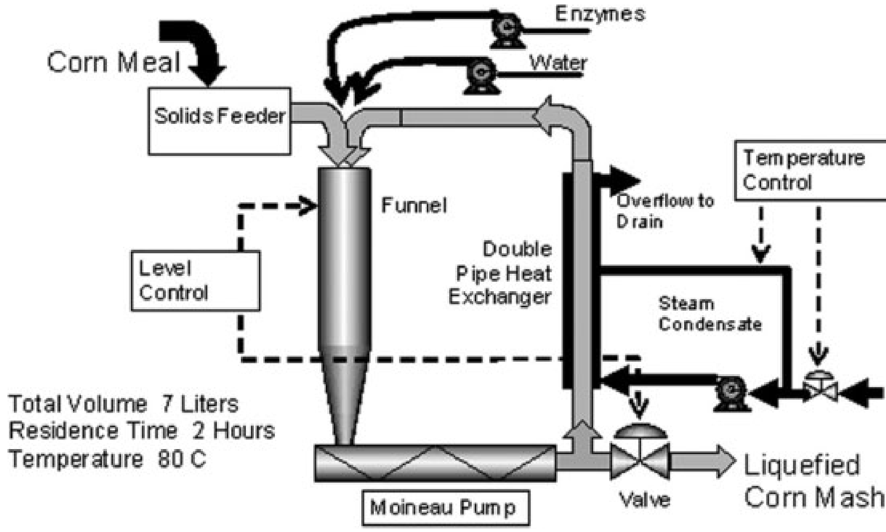

Figure 1. Continuous liquefaction system for high-solids corn mash.

nonfermentable insoluble solids occupy volume and can contribute increased viscosity in the fermentor.[5,6] On the other hand, removing the germ before fermentation reduces the nutrients available to the yeast, and more supplemental nutrients than in whole corn fermentation may be required.[7] A patent has been assigned on the use of anhydrous ammonia to improve the separation of coproducts before fermentation.[8]

It has been reported that the 72-h yield of ethanol from corn treated with 1.0 or 2.0% ammonia for 2 weeks was lower than from untreated corn.[9] The most important measures of fermentability are the yield, rate, and final ethanol concentration. Ethanol concentration and yield are equivalent measures only when compared with the same initial concentration of fermentable substrate. In the fermentation process, high concentration lowers the cost of product and coproduct dewatering operations, but the rate is slower. To optimize the overall cost, it is necessary to know how the rate changes with concentration. The objective of the experiments described here was to compare the rate and yield of fermentation of ammoniated corn with that of nonammoniated corn, and to understand the relationship between rate and yield. Additionally, it was necessary to know if the extra nitrogen in ammoniated corn was available to satisfy the yeast nutritional requirement for nitrogen.

Experimental Methods

Yellow dent corn was obtained from Archer Daniels Midland, Decatur, IL and 400 lb (180 kg) was ammoniated by exposure to anhydrous ammonia in a pilot-scale continuous ammoniator as previously described.[10] Starch and ammonia in corn were measured before and after ammoniation using an enzymatic procedure (Total Starch Analysis Procedure, Megazyme, Bray, Wicklow, Ireland). The ammoniated corn contained 1 g added ammonia nitrogen per kg dry corn. Ammoniated corn was ground the same day in a toothed disk mill (Model LV15K, Glenn Mills, Clifton, NJ).

To make ammoniated or nonammoniated corn mash for 12-L fermentations, 19.1 kg of ammoniated (14.2% moisture) or nonammoniated (11.1% moisture) corn meal was used to make 47 L of mash in a 70-L fermentor (ABEC, Bethlehem, PA). Corn meal and water were mixed and the pH was adjusted to 6.0 with sulfuric acid. Then 11.5-mL Genencor Spezyme Ethyl was added and the mash heated and held at 80°C for 30 min. The mash was cooled and 2.6 kg of corn steep liquor was added to provide ~1.0 g/L FAN. Added ammonia in ammoniated corn provided ~0.3 g/L additional FAN. Mash was adjusted to pH 4.5, and 11.5-mL Genencor Distillase L-400 was added. Distillase L-400 does not have proteolytic activity and does not provide any FAN. The mash was held at 55°C for 2 h. The final volume was adjusted to 47 L with water, divided into four approximately equal batches, and stored in the freezer. Each batch of ammoniated or nonammoniated corn mash was thawed and 2 L of water was added. Mash was transferred to a 16-L stainless steel bench-top fermentor having a working volume of 12–13 L (Microgen SF-116, New Brunswick Scientific, Edison, NJ), heated to 55°C, and held for 2 h to suppress the growth of contaminating cells. Mash was cooled to 35°C and inoculated. Fermentation was continued with manual sampling twice a day for 3 days.

To make ammoniated or nonammoniated corn mash for 50-mL fermentations, 2.67 kg corn meal (same batches of ammoniated and nonammoniated corn meal that were used for 12-L fermentations) were mixed with 4-L tap water in a batchwise operation of the continuous liquefaction system (Figure 1). The solids feeder was removed and replaced with a colloid mill (Supraton, BWS Technology, Kenosha, WI). A peristaltic pump was used to recirculate the contents of the continuous liquefaction system (initially 4 L of water only) through the Supraton mill while the corn meal was manually added to the mill. The mill effluent was returned to the liquefaction system funnel. The mill incorporated the meal into the mash and substantially reduced the particle size. Then the peristaltic pump and Supraton mill were removed, and the mash contained within the recycle loop of the continuous liquefaction system (approximately 7 L) was processed as a batch. The pH was adjusted to 6.0 with sulfuric acid, 1 mL of Spezyme Ethyl was added, and the mash was heated and held at 80°C for 30 min. The mash was cooled and adjusted to pH 4.5, and 0.5-mL Distillase L-400 was added at 55°C and hydrolysis continued for 1 h. The

Table 1. Experiments to Compare 12-L Fermentations of Ammoniated and Nonammoniated Corn

	Initial	Final (72-h)			
	Dry Corn	Glucose	Ethanol	Yield	Productivity
Experiment	Wt %	g/L	g/L	%	g/L/h
Nonammoniated					
1	27.8	0.5	106	80	1.5
2	27.5	0.6	110	83	1.5
3	27.5	0.3	111	82	1.5
Ammoniated					
1	27.3	0.2	105	86	1.5
2	27.3	0.3	107	90	1.5
3	27.2	0.6	109	89	1.5

Corn steep liquor added at 2.0% dry solids to provide approximately 1.0 g/L free amino nitrogen in all experiments.
Maximum theoretical yield is based on complete conversion of starch (70.5 wt% of dry nonammoniated corn and 67.2% of dry ammoniated corn) to ethanol.

mash was spread on a stainless steel tray and frozen. The frozen sheet was broken into pieces. The frozen pieces were removed from storage as needed for the 50-mL fermentation experiments.

Before fermentation, the total solids in the frozen mash were measured by drying in a convection oven at 105°C overnight. The concentration of dry corn solids in the mash was calculated from total solids using glucose concentration in the mash to correct for water of hydrolysis. To each of eight 125-mL Erlenmeyer flasks, 45 g of frozen mash was added. The corn dry solids concentration in each flask was adjusted by adding different amounts of water. Each experiment was performed in duplicate. To each flask, 0.007-mL Genencor Distillase L-400 was added, except for one experiment in which 0.02 mL Distillase L-400 was added to four of eight flasks. Corn steep liquor was added in the range of ~1–2.5% dry solids (0.5–1.25 g/L FAN) in each flask. Each flask was fitted with a rubber stopper with a short piece of Teflon spaghetti tubing inserted to allow the escape of carbon dioxide but prevent loss of ethanol and water by diffusion. Flasks were warmed in a shaker/incubator, inoculated with 1.0 mL of exponentially growing yeast, and incubated at 35°C for 48 h with periodic weighing. After 50 h incubation, the contents of the flasks were transferred to centrifuge tubes and centrifuged at 15,000 rpm for 30 min. The insoluble solids were weighed and the supernatant density, glucose and ethanol concentrations were measured. The final amount of ethanol was calculated from the final weight, residual solids, and ethanol concentration.

For all fermentations, a standard inoculum, ~1.67% v/v exponentially growing *Saccharomyces cerevisiae*, ATCC 4126 (American Type Culture Collection, Manassas, VA) in Difco YM Broth (Becton Dickinson, Sparks, MD) was used as previously described.[11] Glucose and ethanol in samples from all fermentations were measured with a Biochemistry Analyzer (YSI Model 2700, Yellow Springs, OH). The yield was calculated as the percent of complete theoretical stoichiometric conversion to ethanol of the starch in the ammoniated corn (67.2% w/w dry basis) or nonammoniated corn (70.5% w/w dry basis). Ammonia nitrogen in ammoniated corn was measured using an ammonia analyzer (Model TL-200, Timberline Instruments, Boulder, CO).[10] Process cost analysis was conducted using a previously developed computer simulation of a 40 million gallon per year dry-grind ethanol plant.[12]

Results and Discussion

Results for 12-L fermentations of ammoniated and nonammoniated corn are shown in Table 1. The mashes were prepared identically, but the nonammoniated corn mash had a slightly higher concentration because the ammoniated corn had higher moisture content. Ammoniated corn had a lower starch content (67.2% w/w dry basis) when compared with the nonammoniated corn (70.5% w/w dry basis), but the final ethanol concentrations were almost the same for both ammoniated and nonammoniated corn. Because the ethanol yield was calculated based on the starch content of the ammoniated or nonammoniated corn, the ethanol yield was higher for ammoniated than for nonammoniated corn. The higher yield could be explained by the possibility that ammoniation may have weakened the structure of the kernel so that liquefaction and saccharification after coarse grinding released a higher percentage of starch from ammoniated corn than from nonammoniated corn. It is also possible that some starch was destroyed by reaction with anhydrous ammonia. The data indicate that the starch content of ammoniated corn was 5% less than nonammoniated corn. Process simulation and cost analysis showed that the cost of ethanol production is \$0.02 per liter (\$0.07 per gallon) higher from corn containing 5% less starch.

Results from 50-mL shake-flask experiments are shown in Table 2 and Figures 2 and 3. Ethanol yield in 50-mL fermentations was generally higher than in 12-L experiments because the Supraton mill reduced the particle size sufficiently to allow the cooking and amylase treatment to more completely convert the starch to glucose. In experiment 1 (Table 2 and Figure 2), the final concentration and yield of ethanol were compared at different concentrations of nonammoniated corn. As the corn dry solids concentration was increased from 26.7% w/w to 30.3% w/w, the final ethanol concentration increased from 118 to 127 g/L, but the ethanol yield decreased from 93% to 84%, because at the higher corn concentrations, the fermentation did not finish with residual glucose remaining. It can be concluded that to maximize concentration and yield, the optimum is approximately 28% corn dry solids.

In experiment 2 (Table 2 and Figure 3), the final ethanol concentration and yield from nonammoniated corn were compared at different concentrations of corn steep liquor dry solids. As the corn steep liquor dry solids increased from 1 to 2.5%, the final ethanol concentration increased from 118

Table 2. Experiments to Compare 50-mL Fermentations of Ammoniated and Nonammoniated Corn

			Initial		Final (50-h)			
			Dry Corn	CSL	Glucose	Ethanol	Yield	Productivity
Expt.	Flask	A or N	Wt %	DS %	g/L	g/L	%	g/L/h
Different Initial Nonammoniated Corn Concentration								
1	A,B	N	30.4	2.66	16.4	126.8	84.4	2.5
	C,D	N	29.1	2.55	3.7	126.2	89.4	2.5
	E,F	N	27.9	2.44	0.7	124.4	93.1	2.5
	G,H	N	26.7	2.34	0.6	118.3	92.8	2.4
Different Corn Steep Liquor Dry Solid Concentration								
2	A,B	N	28.2	0.99	4.5	117.6	86.4	2.4
	C,D	N	28.2	1.48	2.6	122.7	90.0	2.5
	E,F	N	28.1	1.97	0.3	123.7	90.5	2.5
	G,H	N	28.1	2.46	0.3	124.6	91.2	2.5
Different Initial Ammoniated Corn Concentration								
3	A,B	A	29.3	1.98	2.7	120.8	87.8	2.4
	C,D	A	28.0	1.89	0.8	112.4	86.6	2.2
	E,F	A	26.8	1.81	0.6	113.1	91.2	2.3
	G,H	A	25.6	1.74	0.4	105.6	90.5	2.1
Effect of Extra Glucoamylase								
4	A,B	A	28.4	1.91	13.1	117.7	89.1	2.4
	C,D	A	26.9	1.82	0.9	115.0	92.6	2.3
	*E,F	A	28.3	1.91	9.8	117.3	89.5	2.3
	*G,H	A	26.9	1.82	0.7	116.2	94.2	2.3
Ammoniated and Nonammoniated CSL Requirement								
5	A,B	N	28.1	1.84	1.0	123.1	90.5	2.5
	C,D	N	28.2	1.17	8.0	119.5	87.7	2.4
	E,F	A	26.8	1.81	0.4	115.8	93.5	2.3
	G,H	A	26.9	1.15	0.3	114.8	92.9	2.3

* Added 0.02 mL Distillase L-400 at inoculation, doubling glucoamylase concentration. A, Ammoniated corn; N, Nonammoniated corn; CSL, Corn steep liquor; DS, Dry solids. All data are averages of two flasks.

Maximum theoretical yield based on complete conversion of starch (70.5 wt % of dry nonammoniated corn and 67.2% of dry ammoniated corn) to ethanol.

2.0 % CSL DS provides ~1.0 g/L free amino nitrogen.

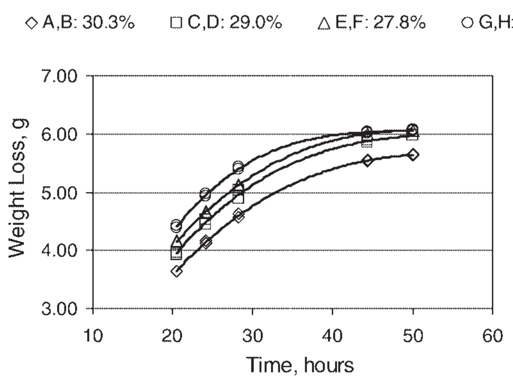

Figure 2. Fifty-mL fermentation of nonammoniated corn.

Weight loss at different corn dry solids (w/w) concentrations. Each flask contained the same amount of corn dry solids. The amount of water and total volume were varied to adjust the concentration for each condition (Experiment 1, Table 2).

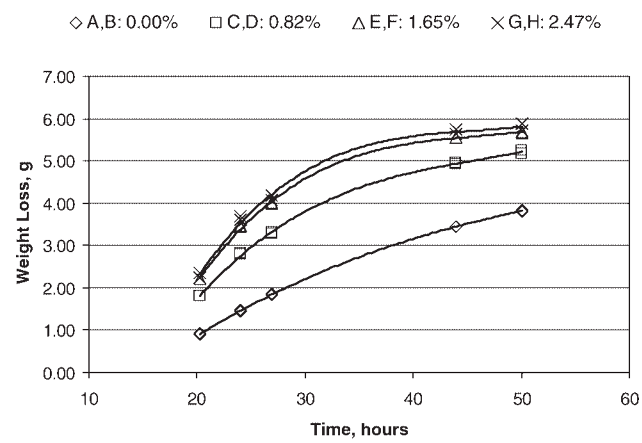

Figure 3. Fifty-mL fermentation of non-ammoniated corn.

Weight loss at different corn steep liquor dry solids (w/w) concentrations. Concentration of corn dry solids (28% w/w) and total volume were the same in each flask (Experiment 2, Table 2).

to 125 g/L and the ethanol yield increased from 86 to 91%. The increased concentration and yield were most apparent between 1 and 1.5% corn steep liquor and the effect was less pronounced between 1.5 and 2.5%. It can be concluded that the optimum condition for 50-h, nonammoniated corn fermentation is ~2% corn steep liquor dry solids.

In experiment 3, the final ethanol concentration and yield were compared at ~2% corn steep liquor dry solids and different concentrations of ammoniated corn. As the ammoniated corn dry solids increased from 26 to 29% w/w, the final ethanol concentration increased from 106 to 121 g/L, but the ethanol yield decreased from 91% to 87%. In experiment 4, conditions from experiment 3 at 27 and 28% ammoniated corn dry solids were repeated with and without addition of extra glucoamylase. It was confirmed that the ethanol yield is higher at 27% than at 28%. Addition of extra glucoamylase increased the final ethanol concentration and yield very slightly.

From experiments 1 through 4, it can be concluded that the optimum concentration of corn dry solids is higher for nonammoniated corn (~28% for nonammoniated corn when compared with ~27% for ammoniated corn). This may be in part because the rate of fermentation of ammoniated corn is less than nonammoniated corn. Also, the final ethanol concentration is less with ammoniated corn than nonammoniated corn in part because the starch concentration in ammoniated corn is less than in nonammoniated corn. From experiments 1 through 5, it can be further concluded that at optimum conditions, the ethanol yield from ammoniated corn is approximately the same as from nonammoniated corn (~91–93%).

Experiment 5 was designed to show that the extra FAN in ammoniated corn could substitute for some of the added corn steep. The amount of extra nitrogen in ammoniated corn was ~1 g per kg dry corn, or 0.3 g/L in the 50-mL fermentations. According to manufacturers' data, 2.0% corn steep liquor dry solids provided the equivalent of 1.0 g/L of FAN. Experiment 2 showed that at 1.0 g/L FAN or higher, the amount of FAN had little effect, but below 1.0 g/L FAN, the amount of FAN had a significant impact on the fermentation. For experiment 5, both ammoniated corn at 27% dry solids and nonammoniated corn at 28% dry solids were compared at two concentrations of corn steep liquor dry solids, 1.8% and 1.2%, providing ~0.9 g/L and 0.6 g/L FAN, respectively. These concentrations were carefully chosen so that the difference would be significant in the fermentation of nonammoniated corn, but the extra 0.3 g/L FAN with ammoniated corn would bring the FAN to 1.2 g/L and 0.9 g/L, where the difference would be much less significant. The data (experiment 5, Table 2) in fact indicate that in the fermentation of nonammoniated corn, the final ethanol concentration and yield were significantly higher at 1.8% corn steep liquor dry solids than at 1.2%, but with ammoniated corn, the final ethanol concentration and yield were only slightly higher at 1.8% than at 1.2%. It can be concluded that the extra nitrogen in ammoniated corn can satisfy at least part of the yeast requirement for FAN, reducing the need for added corn steep liquor.

In 50-mL fermentations with little or no glucose remaining, the highest final ethanol concentration that could be achieved with nonammoniated corn was about 123 to 124 g/L (experiment 1, flasks E,F; experiment 2, flasks E,F and G,H; and experiment 5, flasks A,B), but the highest final ethanol concentration with ammoniated corn was less, about 113 to 116 g/L (experiment 3, flasks E,F; experiment 4, flasks C,D and G,H; and experiment 5, flasks E,F and G,H). This difference may be due to slower fermentation with ammoniated corn.

Conclusions

The measured starch was 5% lower in ammoniated corn than in nonammoniated corn. Comparison of results from 50-mL shake flasks with 12-L fermentor runs was complicated by the fact that the corn solids concentration in the 12-L runs was not controlled as consistently as in the 50-mL experiments. However, the 12-L runs did help to show that saccharification of coarsely ground ammoniated corn may be more complete than without ammoniation. Fermentation of ammoniated corn may be slower provided that sufficient FAN is present in the nonammoniated control. Without any other source of FAN, the optimal amount of corn steep liquor dry solids is ~2.0% w/w in nonammoniated corn fermentations, but less with ammoniated corn, because the additional ammonia nitrogen in ammoniated corn can satisfy at least some of the FAN requirement of yeast. Although ammoniated corn may contain less starch than nonammoniated corn, ammoniation could still be cost-effective in the dry-grind process for fuel ethanol if the cost benefit derived from improved front-end fractionation of ammoniated corn amounts to greater than $0.02 per liter ($0.07 per gallon).

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